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LOW-INTENSITY REPETITIVE MAGNETIC STIMULATION LOWERS ACTION POTENTIAL THRESHOLD AND INCREASES SPIKE FIRING IN LAYER 5 PYRAMIDAL NEURONS *IN VITRO*

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Abstract—Repetitive transcranial magnetic stimulation (rTMS) has become a popular method of modulating neural plasticity in humans. Clinically, rTMS is delivered at high intensities to modulate neuronal excitability. While the high-intensity magnetic field can be targeted to stimulate specific cortical regions, areas adjacent to the targeted area receive stimulation at a lower intensity and may contribute to the overall plasticity induced by rTMS. We have previously shown that low-intensity rTMS induces molecular and structural plasticity *in vivo,* but the effects on membrane properties and neural excitability have not been investigated. Here we investigated the acute effect of low-intensity repetitive magnetic stimulation (LI-rMS) on neuronal excitability and potential changes on the passive and active electrophysiological properties of layer 5 pyramidal neurons *in vitro*. Whole-cell current clamp recordings were made at baseline prior to subthreshold LI-rMS (600 pulses of iTBS, $n = 9$ cells from 7 animals) or sham ($n = 10$ cells from 9 animals), immediately after stimulation, as well as 10 and 20 min post-stimulation. Our results show that LI-rMS does not alter passive membrane properties (resting membrane potential and input resistance) but hyperpolarises action potential threshold and increases evoked spike-firing frequency. Increases in spike firing frequency were present throughout the 20 min post-stimulation whereas action potential (AP) threshold hyperpolarization was present immediately after stimulation and at 20 min post-stimulation. These results provide evidence that LI-rMS alters neuronal excitability of excitatory neurons. We suggest that regions outside the targeted region of high-intensity rTMS are susceptible to neuromodulation and may contribute to rTMS-induced plasticity.

Key words: low-intensity rMS, action potential threshold, spike firing frequency, Intermittent Theta Burst Stimulation.

INTRODUCTION

Repetitive transcranial magnetic stimulation (rTMS) is a popular form of non-invasive brain stimulation used to induce neural plasticity in both clinical and non-clinical populations. rTMS delivers trains of magnetic fields over the scalp which in turn induce electrical currents in the underlying brain. The high-intensity magnetic fields delivered are of the same magnitude of MRI scanners (> 1T) (Ridding and Rothwell, 2007) and can be targeted to stimulate specific brain regions (e.g. motor cortex) and to alter neuronal excitability (e.g. corticospinal excitability). The onset of rTMS-induced changes in corticospinal excitability occurs immediately after stimulation and the effects persist for minutes to hours after stimulation (Huang et al., 2005; Ziemann et al., 2008; Wischnewski and Schutter, 2015). The mechanisms underlying rTMS neuromodulation are unclear, but are believed to involve changes in neuronal membrane properties (Hoppenrath et al., 2016), synaptic and non-synaptic mechanisms (Tang et al., 2015).

While specific regions can be targeted, such that the maximal current induced occurs at the targeted region, regions adjacent also receive stimulation with weaker induced electrical currents and the spread of electrical current from the targeted region (Wagner et al., 2009). The role of low-intensity stimulation in the overall rTMS-induced plasticity remains unclear but studies using extremely low magnetic fields (~0.002 T) have shown changes to neurophysiology (for a review see (Di Lazzaro et al., 2013)) and possibly to cortical excitatory neurotransmission (Capone et al., 2009). In mouse models, we have previously shown that low-intensity rTMS (0.01 T) induces molecular and functional plasticity (Rodger et al., 2012; Makowiecki et al., 2014).

Experimental models of repetitive magnetic stimulation (LI-rMS) using organotypic tissue cultures or brain slices from animals provide a useful adjunct to human studies as they allow direct measurement of

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Abbreviations: ACSF, artificial cerebrospinal fluid; AHP, after hyperpolarization; AP, action potential; LI-rMS, low-intensity repetitive magnetic stimulation; RMP, resting membrane potential; rTMS, repetitive transcranial magnetic stimulation.

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plasticity at the single-cell level and provide insights into the cellular changes occurring after rTMS (for a review see (Müller-Dahlhaus and Vlachos, 2013; Tang et al., 2015)). Single-cell electrophysiological studies on brain slices of rats that received high-intensity rTMS show changes in the resting membrane potential and evoked spike firing of layer 2/3 fast spiking interneurons two hours after stimulation (Hoppenrath et al., 2016). However the effects of LI-rMS on the electrophysiological properties of cortical excitatory neurons are unknown. To investigate these effects, we employed *in vitro* whole-cell patch clamp electrophysiology on layer 5 pyramidal neurons from mouse motor and somatosensory brain slices. We investigated both passive and active membrane properties and evoked spiking properties following LI-rMS or sham stimulation over a 20-min period post-stimulation. Our results show that LI-rMS does not alter passive membrane properties but increases neural excitability by inducing hyperpolarized action potential thresholds and increases the evoked spike firing rate.

EXPERIMENTAL PROCEDURES

Ethics approval

All procedures were approved by the University of Western Australia animal ethics committee (RA/3/100/1229) which is in accordance with the Australian code of practice for the care and use of animals for scientific purposes.

Slice preparation

C57Bl/6J mice (post-natal days 12–15, of either sex, $n = 11$) were acquired from the Animal Resource Centre (Murdoch, Australia). Juvenile animals were chosen due to the high quality and longevity of the slices that they provide. Mice were terminally anaesthetized with an intra-peritoneal injection of pentabarbitone (>160 mg/kg) followed by rapid dissection of the brain. Acute brain slices (300 μm thick) were prepared from the motor and somatosensory cortex. Coronal slices of cortex were prepared with a vibrating slicer (Campden Instruments 5000-mz) and ice-cold cutting solution comprising (mM) 125 NaCl, 3 KCl, 0.5 $CaCl_2$, 6 $MgCl_2$, 25 $NaHCO_3$, 1.25 NaH_2PO_4 and 10 glucose bubbled with carbogen (5% CO_2/95% O_2). Slices were kept at 35 °C for 1 h in a holding chamber containing carbogen-bubbled artificial CSF (artificial cerebrospinal fluid (ACSF), see below for composition), after which they were held at room temperature until required.

Electrophysiology

Slices received continuous perfusion (~1.5 mL/min) with ACSF comprising (mM) 125 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 25 $NaHCO_3$, 1.25 NaH_2PO_4 and 25 glucose bubbled with carbogen and maintained at 35 ± 2 °C (Warner Instruments TC-324B). For whole-cell current clamp recordings 6-10 MΩ borosilicate glass patch electrodes (Harvard apparatus GC150F-15, 1.5 mm outer dimeter × 0.86 inner diameter, SDR scientific, Australia) were filled with an internal solution comprising (mM) 135 potassium gluconate, 10 HEPES, 7 NaCl, 2 Na_2ATP, 0.3 Na_3GTP, 2 $MgCl_2$.

Slices were visualized at 40× magnification under bright field and infrared differential interference contrast video microscopy (Olympus BX51-WI). Somatic recordings were made using a Multiclamp 700B (Axon Instruments) and digitized with a Digidata 1440A, under the control of Axograph (Axograph X 1.5.4) and data acquired at a sampling rate of 50 kHz.

Whole-cell current clamp recordings were conducted on layer 5 (L5) pyramidal neurons. As we hypothesized *a priori* that stimulation would alter neuronal excitability and membrane properties, whole-cell recordings were made without applying holding currents during experimental procedures.

To investigate action potential (AP) properties (AP threshold, spike rise time, spike height, fast after hyperpolarization), single AP's were evoked with a 5-ms long depolarizing current step of +800 pA (Fig. 1D), repeated every second for a total of 10 s (i.e. 10 single AP's per recording).

To investigate the spike firing properties, spikes were evoked with an AP family protocol (Fig. 1C) consisting of 500-ms current steps ranging from −200 to +500 pA (20 current steps, with a 30-second interstep interval), which was repeated once more after a 30-s delay.

Cells were discarded and excluded from analysis if the series resistance changed by >20% of baseline value and/or exceeded 30 MΩ. Current clamp bridge balance was adjusted prior to each AP family and single AP recording.

Repetitive magnetic stimulation (LI-rMS)

The LI-rMS protocol delivered was iTBS (Huang et al., 2005) (Fig. 1B) and consisted of trains of three 50-Hz pulses, repeated every 200 ms for 2 s. Trains were repeated once every 10 s for a total of 20 repetitions (total of 190 s). Monophasic pulses (400 μs rise time) were delivered with a custom circular coil (described in (Tang et al., 2016) (8 mm outer diameter, with an iron core). Coils were fixed to an electronic micro-manipulator and positioned in-between the slice chamber and microscope condenser (Fig. 1A). The coil was placed at a distance of approximately 1 mm from the slice, with the coil edge placed below the cortical layers; therefore apical dendrites from layer 5 pyramidal neurons were oriented perpendicular to the coil. The peak magnetic field at a distance of 1 mm from the base of the coil was measured with a Hall-effect probe (Honeywell, SS94A2D, USA) to be 85.4 mT and had a dB/dT of ~285 T/s. Coils were controlled by an arbitrary waveform generator (Agilent 33551B, Measurement innovation, Australia) and a programmable DC power supply (Kepco BOP 100-4 M, TMG test equipment, Australia). Sham stimulation consisted of placing a coil detached from the power supply beneath the slice as described above for 190 s before beginning the post-stimulation$_{+0}$ recordings.